

# MIF Inhibition Enhances Axon Regeneration after Spinal Cord Injury

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Finally, to my family: Amma, Appa, and Suwetha, I love you more than anything.

## **Abstract**

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that regulates a portion of the immune response and that has been implicated in the inhibition of neuronal recovery following spinal cord injury. My hypothesis is that MIF inhibits neuronal recovery by increasing the inflammatory response as well as the buildup of scar tissue. To test this hypothesis, MIF was inhibited in the lamprey after spinal cord transection via administration of the drug ISO-1, a small protein that inhibits the enzymatic activity of MIF. The behavioral recovery was then measured over thirteen week period by scoring swimming patterns. Axonal regeneration was measured through retrograde labeling of the somata of giant reticulospinal axons. Results indicate that MIF inhibition led to a 60.6% increase in axon regeneration, as well as more complete behavioral recovery. These results suggest the possibility of the MIF inhibition being a viable treatment for neural recovery following spinal cord injury.

## **Introduction**

### **The Vertebrate Nervous System**

The vertebrate central nervous system is a rich, complex system that processes information taken in from the environment and relays commands to the rest of the body to ensure the coordination of organs and body parts, behavior, and consciousness. The nervous system comprises two main parts – the central and the peripheral nervous system (Jacobson 2007). The central nervous system is comprised of the brain and spinal cord, and regulates coordination between different systems. The peripheral nervous system acts as the link between the CNS and the rest of the body, and is responsible for tasks such as retrieving sensory information, and enervating muscles to cause movement. Nerve cells, called neurons, are contiguous cells that relay information through a series of electrical impulses. The neuron is made up of three parts, the soma, the axon, and the synapse (Jacobson 2008). Somata contain the nucleus and other organelles. Synapses are the physical junctions between axons, long projections from the soma, and dendrites located on the soma of other neurons. The neurons of the central nervous system are surrounded by glial cells. Glial cells are non-neuronal cells that have a variety of functions, providing structural, metabolic, signaling and immune support for the neurons (Jacobson 2008).

Damage to any part of the nervous system, however, can lead to disruption in the neural network that can adversely affect systemic processes such as metabolic functions and

regulation. Signaling, movement, and sensory perception can all be impaired. Injury to the central nervous system, and especially the spinal cord, can leave irreversible damage.

## **Spinal Cord Injury**

Spinal cord injury can leave devastating consequences due to its disruption of vital systemic processes including behavior and locomotion, and a sizable proportion of the population suffers from the effects. The most recent results from the National Spinal Cord Injury Statistical Center (University of Alabama, Birmingham) reports the annual incidence of new spinal cord injury victims at approximately 12,000. The United States alone has about 255,702 people who have suffered from the affliction. Though spinal cord injury may seem far removed from the realm of everyday life, it is in fact a very real possibility. Almost half the cases are due to vehicular accidents, with sports and falls as other common factors. Added to the fact that the average age of injury is reported to be 39.5 years, it is clear that not only can spinal cord injury potentially affect a large portion of the population, but it can also affect one's quality of life for several decades thereafter.

The effects of spinal cord injury are numerous and varied, and can affect the individual physically and psychologically. The most evident and well-known consequences of spinal cord injury are paraplegia (when only the lower body is affected) and tetraplegia (the upper body and arms also display evidence of impaired function). When trauma is sustained at the spinal cord, neurons governing the sensory and motor functions of the lower body are damaged,

leading to loss of sensation and voluntary movement, or sometimes very jerky, uncontrollable movement below the site of damage. Severed axons disrupt signaling between neurons above and below the injury site. Axons above the lesion begin to retract towards the brain. Axons below begin to degenerate, resulting in a permanent impairment and possible loss of signaling to areas below the lesion. In the worst case, complete paralysis occurs, leaving the victim with greater obstacles in maintaining their independence. But there are also other associative complications reported consistently. On a purely physical level, victims find themselves suffering from urinary tract infections, spasticity, pressure ulcers, and deep vein thrombosis (Calandra 2003). On a psychological level, suffering, pain, and loss of independence can be responsible for a high incidence of depression, which only serves as yet another obstacle to possible rehabilitation.

Part of what makes rehabilitation for spinal cord trauma so tricky and frustrating is the need to address issues on two fronts. For one, the injury itself must be healed. For example, the tissue damage incurred at the site of impact must be repaired, and the cellular integrity must be restored. But the recoupment of function further depends on the regeneration of the neurons that were wounded and destroyed. Severed axons between the brain and neurons beyond the injury site cause neuron death, the cause of paralysis. Neurons must be able to grow again across the injury site and reform connections in order for movement and function to be regained.

## **Wound Healing and Macrophage Migration Inhibitory Factor (MIF)**

Recovering from any injury requires a careful, delicate balance in the wound healing process. Damaged tissue must be healed, structure must be repaired, and foreign matter that may infect the wound must be removed. However, an overexpression of the immune response can lead to inflammation, which comes with several negative consequences, including a number of common diseases, including sepsis, arthritis, diabetes, and atherosclerosis (Bennermo, et al. 2004; Dandonna et al. 2005).

After sustaining an injury to any part of the body, including the spinal cord, the immune system in vertebrates is triggered. Various cellular components, including macrophages, microglia, and neutrophils present in the surrounding tissue are activated with spinal cord lesion and damage to the surrounding blood vessels and tissue (Jones et al., 2005). These immune cells migrate to the injury site to isolate and eliminate pathogens, and to produce pro-inflammatory cytokines and chemokines. Cytokines and chemokines, in addition to upregulating immune cell presence at the injury site, also cause vasodilation, edema, and initiate the formation of scar tissue. This inflammatory response in vertebrates is the initial stage of the wound healing process, and it is triggered within minutes of the infliction (Calandra 2003).

The cytokine central to our study is macrophage migration inhibitory factor (MIF), which serves many different functions throughout the vertebrate system, including as a pituitary hormone and migration inhibitor (Bucala et al. 1996; Nishio et al., 2008). MIF is a cytokine secreted in the central nervous system by immune cells such as microglia, astrocytes, and

ependymal cells. It is highly conserved across vertebrate organisms (Nishio et al., 2008). As a pro-inflammatory cytokine, MIF recruits and maintains the presence of immune cells that include, in addition to macrophages, microglia and lymphocytes at the injury site (Bucala et al. 1996). MIF mRNA, following injury to the spinal cord, is upregulated at the wound site, setting off the immune response (Nishio et al., 2008). The increased presence of MIF can lead to overexpression of the immune response. MIF and the amassment of immune cells comprise a feed-forward mechanism that can result in debilitating inflammation.

MIF is thought to be a hindrance to the wound healing process for a number of different reasons. Through its partial control over the inflammatory response, MIF has been implicated in the activation of several pro-inflammatory pathways, as well as in the mediation of numerous inflammatory diseases, such as arthritis, sepsis, tissue and neuronal destruction, and even death (Al-Abed et al. 2005). At the inception of our study, the (possibly negative) effects of MIF and inflammation on axonal regeneration were unknown. At a basic level, the sheer volume of immune cells, dead tissue, and foreign matter at a wound site during inflammation can prevent axons from extending through and past the lesion (Jones et al., 2005). On a more pathological level, MIF production also increases in astrocytes after injury (Calandra et al., 2003). Astrocytes are immune cells that form glial scar tissue, which is meant to contain the huge amount of matter present at the lesion as a result of the immune response. Scar tissue can also prevent effect regeneration, altering the structural integrity of the connective tissue and acting as physical barriers to nerve regeneration (Jones et al. 2005). The decrease of MIF partially inhibits scar tissue formation, suggesting that lower levels of MIF may facilitate axonal



growth across the lesion site by leaving a clearer path (Nishio et al. 2008). MIF has been shown to undergo significant up-regulation following spinal cord injury in rats, which implies that decreasing the MIF present directly after spinal cord injury may affect rehabilitation (Hashimoto, 2005). Together, these data implicate MIF as a negative factor for neuron repair and regeneration after spinal cord injury. **Therefore, my hypothesis is that inhibiting MIF directly after spinal cord injury will reduce inflammation and the production of scar tissue, thus leading to greater axonal regeneration and more complete behavioral recovery. To do so I will utilize the lamprey spinal cord, which provides a robust model for recovery after spinal cord injury.**

### **The Lamprey as a Model Organism for Spinal Cord Research**

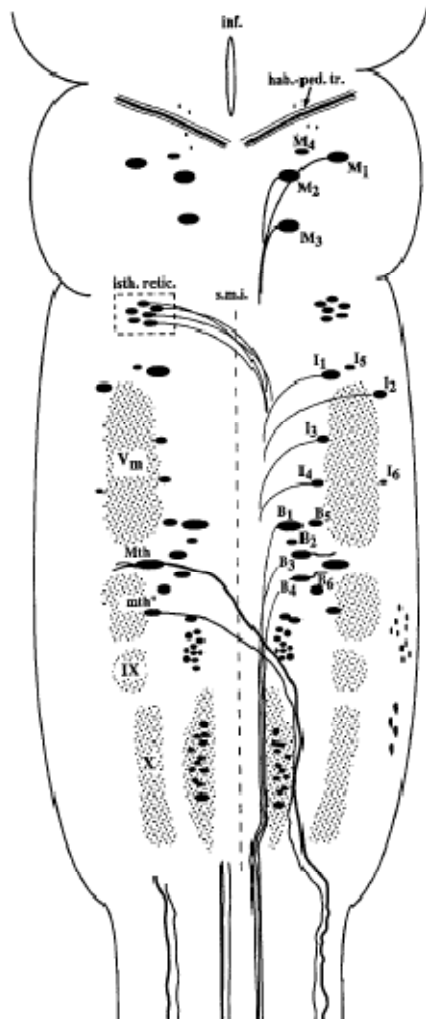
For our studies, we used lamprey as a model organism. The lamprey is the oldest living vertebrate, and the exact species used in this study is *Petromyzon marinus*, a species of sea lamprey (Osório, et al. 2008). The lamprey central nervous system is particularly well designed for neurobiological research. Larval lamprey spinal cords are long and ribbon-like, connecting from the brain and stretching across the length of the organism, directly above the notochord, a primitive vertebrate column. The spinal cord is translucent and made up of numerous smaller axons and several giant reticulospinal axons that travel down the spinal cord without crossing each other. Each axon projects from a cell body in the brain. The axons are somewhat different from other vertebrate axons in that it lacks a myelin sheath, which contributes to its relative

transparency of the spinal cord (Rovainen et al. 1967). The largest group of axons are the giant reticulospinal axons.

### **Lamprey Neuroanatomy**

There are thirty-six giant reticulospinal axons that split into two symmetrical groups on each side. The giant axons are labeled based on their position in the brain, and consist of M cells, I cells, B cells, the Mauthner, and the auxiliary Mauthner (fig. 1) (Swain et al. 1993). The reticulospinal axons descend from the bilateral reticular nuclei of the lamprey brain stem (Orlovsky, 2000; Swain, 1993). They are responsible for initiating the locomotion of the lamprey, maintaining equilibrium, and controlling their characteristic sinusoidal swimming behavior.

**Figure 1: Labeled Cell Bodies of Lamprey Giant Reticulospinal Axons**

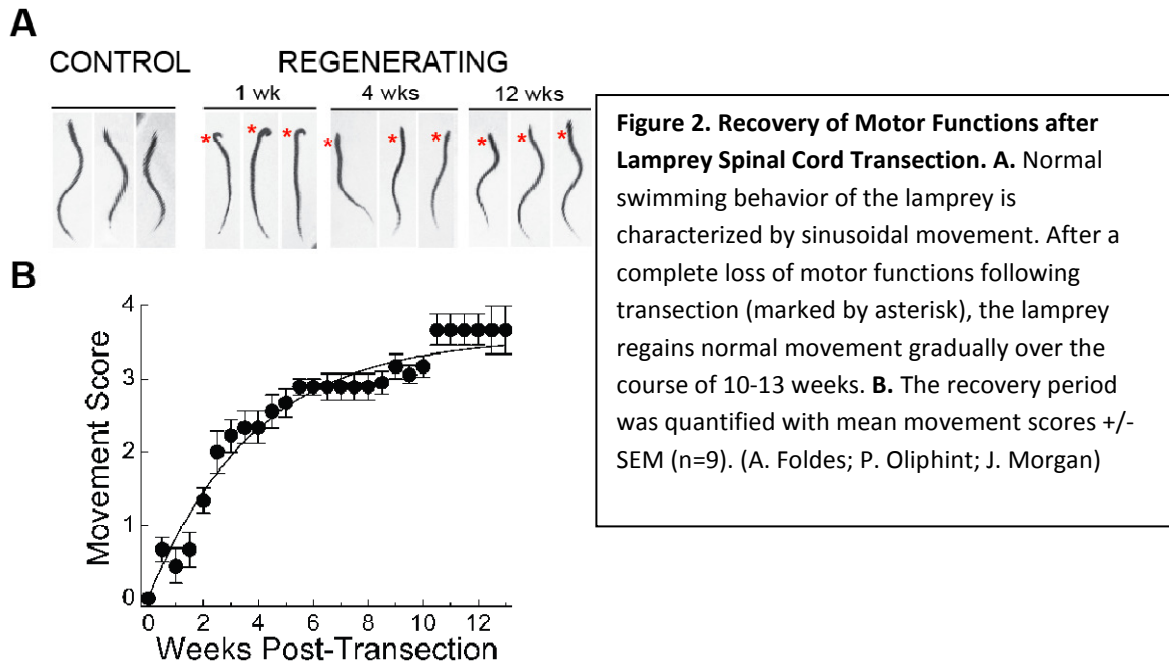


**Figure 1. Labeled Cell Bodies of Lamprey Giant Reticulospinal Axons.** Jacobs et al. 1993. Lamprey giant reticulospinal axons are labeled and arranged in symmetrical groups throughout the brain.

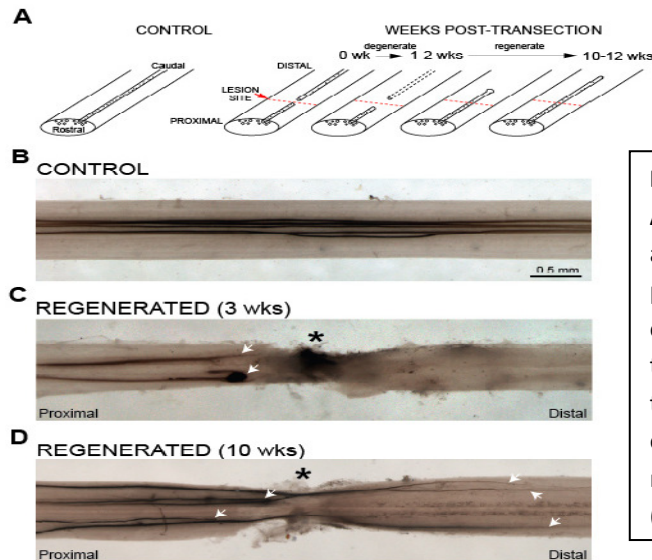
The lamprey is a particularly fitting organism for this study for a number of different reasons. The giant axons are easy to see and can be easily identified through the use of a dissecting microscope. Furthermore, the presence of each axon can be determined through the consistent arrangement of their cell bodies in the brain. The axons can be clearly labeled, allowing for identification throughout an experiment. Most importantly, the lamprey spinal cord axons have the ability to regenerate to some extent - anywhere from 10-95% of normal

locomotive function can be regained (Rovainen et al. 1967). After complete transection of the spinal cord, the severance of the giant reticulospinal axons leads to complete paralysis below the lesion site. However, the lamprey recovers from paralysis and regains movement after a period of ten to thirteen weeks (figure 2). Anatomical investigation (figure 3) shows that the reticulospinal axons and synapses regenerate across the lesion site, reforming crucial connections, and this time course of regeneration correlates with the timing of behavioral recovery. This suggests that the reticulospinal axons assist to some degree in enabling the lamprey to resume its locomotive capabilities (Rovainen 1976; Selzer, 1978)

**Figure 2: Recovery of Motor Functions after Lamprey Spinal Cord Transection**



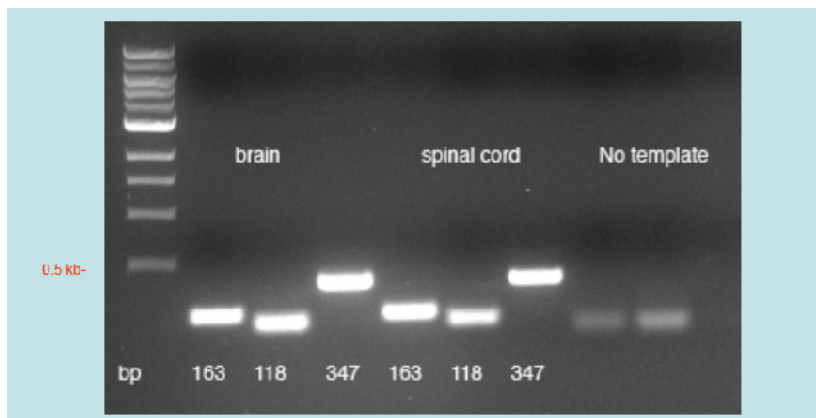
**Figure 3: Regeneration of Giant Reticulospinal Axons across Transection Site**



**Figure 3. Regeneration of giant Reticulospinal Axons across transection site. A.** Degeneration and Regeneration of RS axons over a 12-week period. **B.** Control spinal cord. **C.** Regeneration of RS neurons 3 weeks after spinal cord transection. Asterisks in C and D indicate site of transection. White arrows indicate terminal ends of giant RS axons. **D.** Regeneration of RS neurons 10 weeks after spinal cord transection. (P. Oliphint; B. Lau; J. Morgan)

In addition to the characteristics of its central nervous system that make the lamprey an excellent model organism for this study, the lamprey also possesses a comparable immune system to other vertebrates. The lamprey has been shown to use many of the same cytokines as mice and humans, including interleukin-8, interleukin -7, nuclear factor- $\kappa$ B, and most importantly, MIF. Studying the effect of MIF on regeneration after spinal cord injury in the lamprey has excellent potential for a number of different reasons. The coding sequence for MIF is highly conserved between the lamprey and mice, rats, and humans, suggesting a high possibility of conservation of functions as well. In the lamprey, MIF mRNA has been shown to be expressed in the brain and spinal cord (figure 4), and strong expression of the protein itself has been found in the spinal cord.

**Figure 4: MIF Expression in Lamprey Brain and Spinal Cord**



**Figure 4. MIF Expression in Lamprey Brain and Spinal Cord.**

Oligonucleotides against lamprey MIF successfully amplified 183 and 118 bp MIF fragments, and full-length (347 bp) brain and spinal cord cDNA libraries. This is an indication of MIF expression in both the brain and the spinal cord. "No Template" indicates lane where no cDNA was included in the reaction. *O. Bloom; J. Morgan*

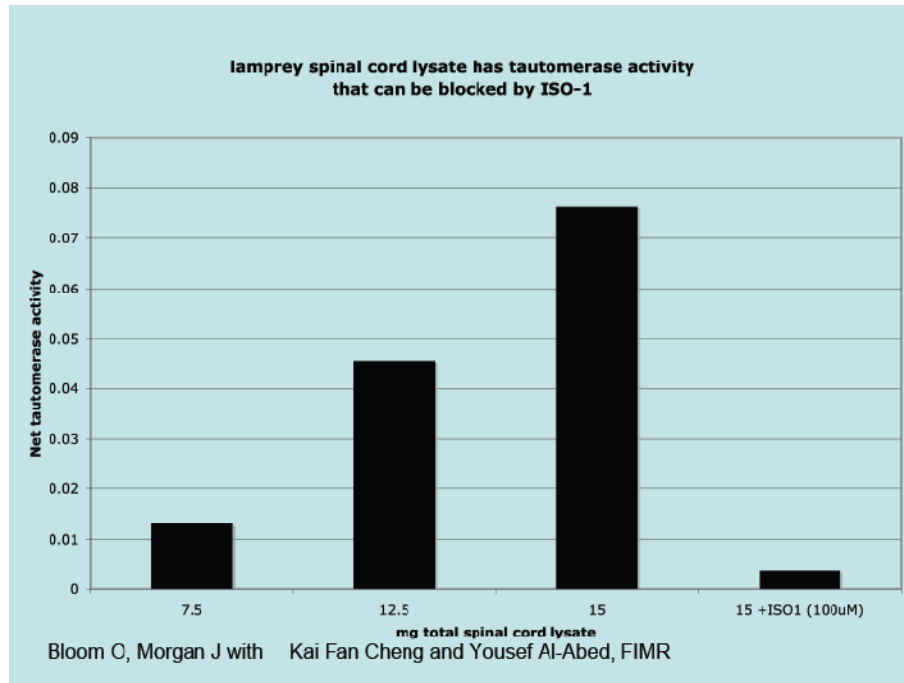
## Supporting Data

### MIF Expression in the Lamprey

MIF has enzymatic activity, which is thought to be regulated by tautomerization, and has been successfully inhibited by tautomerase inhibitors (Rorsman, 1996; Tracey 2005). Inhibition of MIF can be effectively controlled through the use of (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1), a synthetic molecule designed by the laboratory of Dr. Kevin Tracey (Laboratory of Biomedical Science, North Shore-Long Island Jewish Institute for Medical Research). ISO-1 is a small molecule that inhibits the enzymatic

activity of MIF by binding to the active site. It has been shown to effectively inhibit lamprey MIF (figure 5) (Lubetsky et al., 2002).

**Figure 5: Inhibition of Lamprey MIF Activity through ISO-1**



**Figure 5. Inhibition of Lamprey MIF Activity through ISO-1.** Increased tautomerase activity was exhibited with increasing levels of lamprey spinal cord lysate (a stimulant of enzymatic activity). The addition of ISO-1 showed significant inhibition of tautomerase activity. **O. Bloom; J. Morgan**

In this experiment, following spinal cord transection in the lamprey, internal MIF will be inhibited via an application of ISO-1 at the lesion site. A set of control lampreys will be treated solely with a vehicle. Behavioral recovery will be scored over a period of thirteen week, after which the somata of axons that have regenerated across the lesion site will be retrogradely labeled. I expect that the inhibition of MIF following spinal cord injury will curtail the inflammatory response and reduce scar tissue, thus increasing both behavioral recovery and neuron regeneration.



## **Methods**

### **Insertion of ISO-1/Vehicle**

Twelve larval lampreys (labeled alphabetically A through L) underwent full spinal cord transection at the fifth gill, and all were rendered completely paralyzed below the lesion site. Six of the lampreys received applications of 0.1 ml of 10 mg/ml ISO-1 in 30% hydroxypropyl- $\beta$ -cyclodextrin (HBC)/2% dimethyl sulfoxide (DMSO) solution (1 mg/animal). The other six lampreys received only the DMSO vehicle. The drug and the control were applied through a small piece of GelFoam soaked in the appropriate system, which was placed at the lesion site before the wound was sutured.

### **Behavioral Analysis**

The lampreys were left to recover from their transection, with careful monitoring twice a week for approximately 20-30 minutes. With the day of the initial transection as Day 0, each lamprey was observed twice a week on the fourth and seventh days of each week. Each lamprey was allowed to swim in a large tank, and was subject to stimulation through a tail pinch. The swimming behavior of the lamprey was then evaluated on a scale of zero to four.

## Movement Score

- **'0'** – Complete paralysis. Lamprey shows no response to stimuli.
- **'1'** – Lamprey exhibits erratic movement, often characterized by curling into a 'C' or 'S' shaped curve. No swimming is observed.
- **'2'** – Lamprey exhibits erratic movement and highly inconsistent swimming behavior, often in short bursts (1-15 seconds).
- **'3'** – Lamprey has regained some persistent swimming behavior, but movement is still abnormal, and there are often observable kinks in the body.
- **'4'** – Lamprey appears to have regained full sinusoidal movement, moving smoothly and swimming consistently for extended periods of time. The lamprey may be slow to right itself during swimming.

Julie Schackman and I scored the twelve lampreys at separate times for a period of thirteen weeks, taking care not to discuss our individual scores with each other until the end of the experiment. The study was double-blind; the experimenter placed either ISO-1 or the vehicle at the lesion site of each lamprey without knowing what each lamprey received. Neither Julie nor I knew which lampreys had been treated with ISO-1 and which had been treated with only the vehicle until after the 13-week recovery period.

## HRP Labeling

Following the 13 week recovery period, the lampreys underwent an axon labeling procedure. The tracer used was horseradish peroxidase (HRP, Type IV, Sigma), which was taken up retrogradely through the axons to their respective cell bodies in the brain (Swain, et al. 1993). Each lamprey was transected 3 millimeters caudal to the original transection site (the site of ISO-1/vehicle application). A small piece of Gelfoam, roughly  $1\text{mm}^{-2}$  was allowed to soak in 40% HRP solution in lamprey internal solution (180mM KCl, 10mM HEPES, pH 7.4) solution for at least 1 hour in refrigeration. Following a second complete spinal cord transection, the Gelfoam was applied to the lesion site, and the wound was sutured. The cut axons took up the HRP and retrogradely transported it to the somata in the brain over the next week. Thus, this approach will only label axons that regenerated distally to the lesion site.

After 1 week, the lampreys were sacrificed, and the brain and spinal cord were removed. The tissue was reacted in Hanker-Yates reagent (Sigma; 37 mg chromagen in 20 mL cold 0.1 M Tris buffer at pH 7.2, 0.1% hydrogen peroxide) for approximately 5-10 minutes, though some variability is to be expected. The tissue must be kept under careful observation for the duration of the reaction, and the solution should be removed after the cell body staining is a dark purple-brown shade and the background staining begins to darken. The tissue was immediately fixed in 4% paraformaldehyde before storage in 1M PBS.

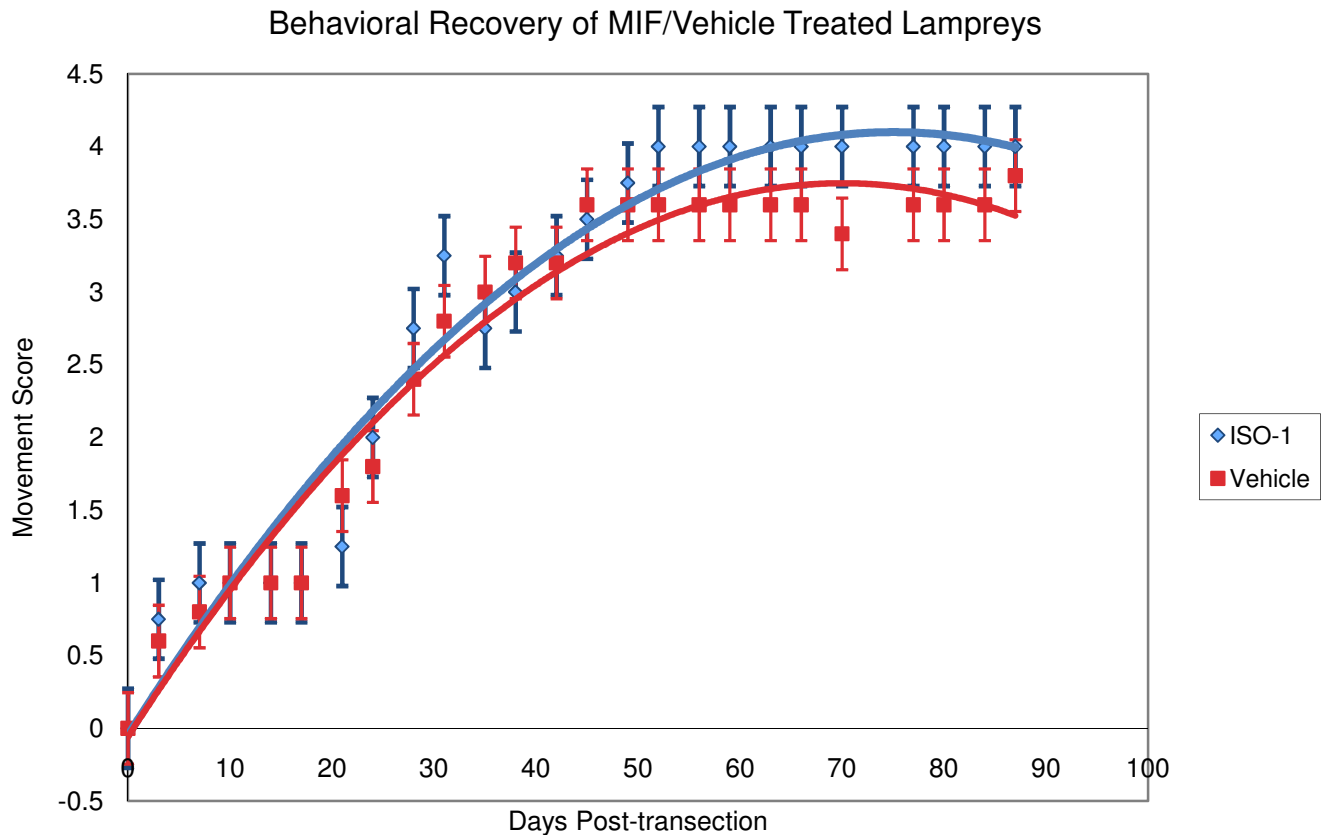
## Results

### **MIF Inhibition Yields More Complete Behavioral Recovery**

To determine whether MIF plays a measurable role in the recovery of lamprey swimming behaviors after spinal cord injury, lampreys were treated with a specific inhibitor of MIF (ISO-1) or with vehicle alone at the time of spinal cord transection. The lampreys were then allowed to recover normally for a 13 week period, and their level of movement was scored according to 0-4 scale described in Methods. At the end of the recovery period, the lampreys were revealed to either have been treated with ISO-1 or with only the vehicle.

The movement scores of all the lampreys over the recovery period were averaged according to whether it had been treated with the MIF inhibitor, ISO-1, or only with the vehicle. Several lampreys were not included in the final due to the possibility of inaccuracy due to effects unrelated to the experimental variables. For example, one lamprey exhibited a growth at the lesion site that caused muscle and tissue to completely choke out the lesion site and preclude any possibility of regeneration.

**Figure 6: Comparison of Recovery Rates**



**Figure 6. Comparison of Recovery Rates.** The rates of recovery for vehicle-treated lampreys (red), +MIF inhibited lampreys (blue).

In examining the data, the lampreys treated with ISO-1 show a slightly higher rate of recovery than the lampreys treated only with the vehicle (figure 6). At maximum recovery, the lampreys treated with ISO-1 show a 5.2% better recovery with a peak score of 4.0 after 56 days of recovery. The lampreys treated only with the vehicle reach 3.8 at 91 days, a much longer interval. At 45 days, which is approximately halfway through the recovery period, the lampreys treated with ISO-1 still exhibit better recovery than their vehicle-treated counterparts, with a

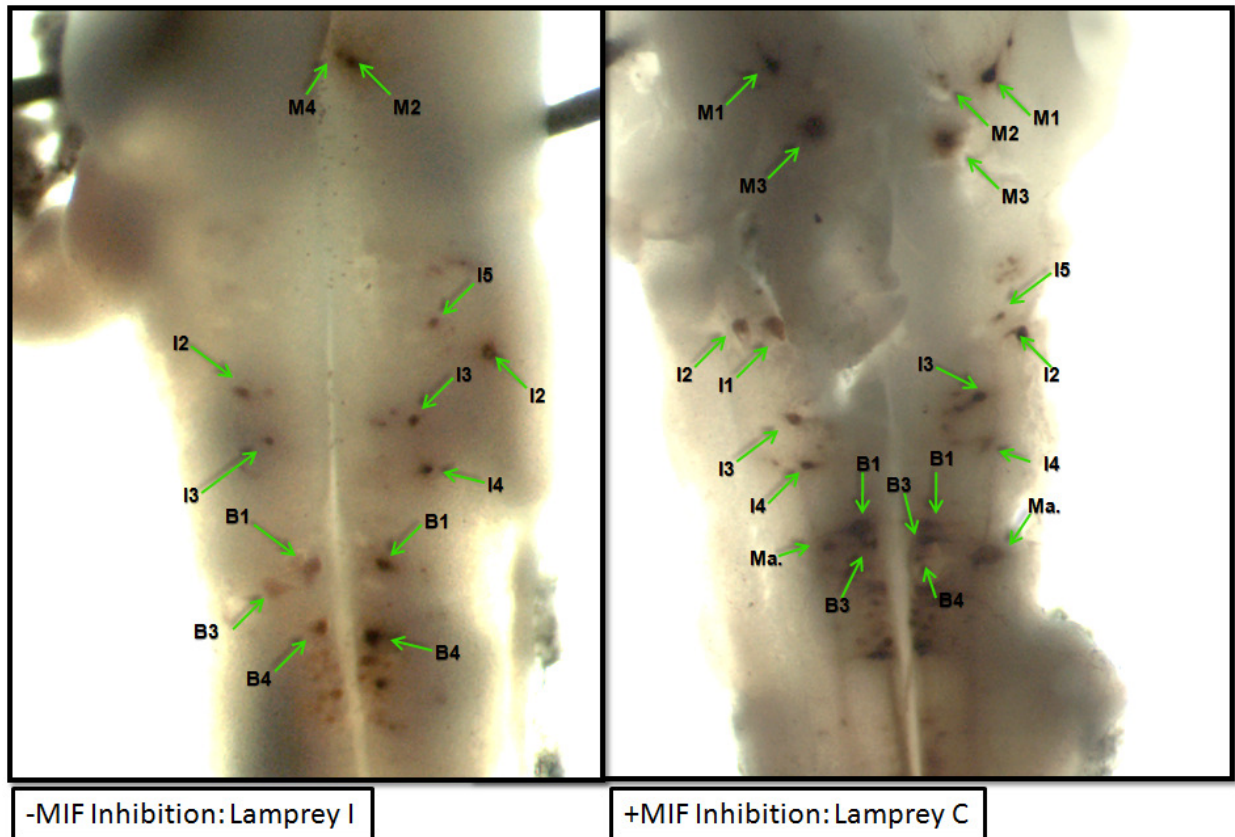
score of 3.25 versus 3.2 (a 1.56% increase). The ISO-1 treated lampreys reach half their maximum score (2.0) after 24 days of recovery. The vehicle treated lampreys reached half their maximum score (1.9) at approximately 25 days.

### **MIF Inhibition Increases Reticulospinal Axon Regeneration**

To determine whether MIF plays a role in reticulospinal axon regeneration, following the behavioral recovery time course, RS axons in each of the 12 experimental lampreys were labeled with HRP using the procedures described in the Methods. The lamprey brains were then labeled with HRP – gelfoam saturated in 40% HRP solution was placed at a transection site 3 mm caudal to the original site of ISO-1/vehicle application. After one week, the brains were then removed from each lamprey and placed in a dish to be reacted with Hanker-Yeats reagent (see Methods), resulting in darkly-stained somata of reticulospinal axons. The somata were counted and the numbers analyzed. The results are summarized in Table 1.

This approach only allows for retrograde labeling of regenerated axons. Therefore, counting the number of giant RS somata labeled with HRP is an indication of how many of these giant axons regenerated across the lesion site. Several lampreys were not included in the final analysis because the RS axons and somata of these lampreys were not successfully labeled with HRP, rendering them unable to be counted and included in the final data totals. Therefore, for the final analysis, I used data from 3 ISO-1 treated lampreys and 3 vehicle treated lampreys.

**Figure 7: ISO-1 enhances the number of HRP-labeled neurons in lamprey brains, indicating greater axon regeneration**



**Figure 7. ISO-1 enhances the number of HRP-labeled neurons in lamprey brains, indicating greater axon regeneration.** Green arrows indicate cell bodies of giant reticulospinal axons that were successfully labeled, thus indicating axonal regeneration across the lesion site. Lamprey I (left) is an example of a lamprey treated only with the vehicle and exhibits 13 somata of regenerated RS axons. Lamprey C (right) is an example of a lamprey treated with ISO-1 and is shown to have regenerated 18 RS axons. A. *Amsavelu*

In lampreys in which MIF was not inhibited and only the vehicle was present, the brain showed a moderate number of labeled somata. Of the lampreys treated with ISO-1 and undergoing MIF inhibition, a higher number of somata of giant reticulospinal axons appeared to be labeled. Figure 7 is side-by-side comparison of examples from each group.

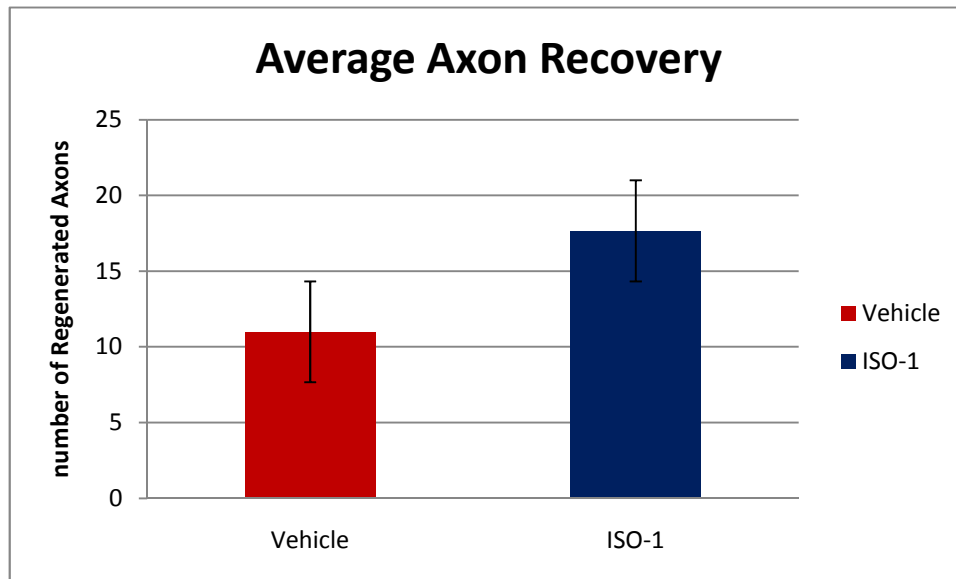
Lamprey I is an example of a lamprey brain treated only with the vehicle. Green arrows indicate the labeled somata, revealing the corresponding reticulospinal axons M2, M4, I2, I3, I4, I5, B1, and B4 on the right side and axons I2, I3, B1, B3, and B4 on the left. These 13 giant reticulospinal axons had regenerated across the lesion site. Lamprey C is an example of a lamprey that had been subject to MIF inhibition, and after reaction, the brain showed labeling of cells M1, M2, M3, I2, I3, I4, I5, B1, B3, and B4, and the Mauthner on the right side, and M1, M3, I1, I2, I3, I4, B1, B3, and the Mauthner on the left. There were a total of 18 axons that had regenerated across the lesion site.

**Table 1: Regenerated Axons**

	Lamprey	Regenerated Axons (Right)	Regenerated Axons (Left)	Total number of Regenerated Axons
<b>+MIF Inhibition (ISO-1 application)</b>	<b>C</b>	M1, M2, M3, I2, I3, I4, I5, B1, B3, B4, Mauthner	M1, M3, I1, I2, I3, I4, B1, B3, Mauthner	20
	<b>D</b>	M2, M3, I2, I3, I4, I5, B1, B3, B5, Mauthner	M2, M3, I2, I3, I4, B1, B2, B5, Mauthner	19
	<b>G</b>	M2, M3, M4, I2, I3, B4, Auxiliary Mauthner	M1, M3, M4, I3, I4, B2, B4,	14
<b>-MIF Inhibition (ISO-1 application)</b>	<b>A</b>	M2, M4, I1, B1, B5, Auxiliary Mauthner,	I1, I3	8
	<b>I</b>	M2, M4, I2, I3, I4, I5, B1, B4	I2, I3, B1, B3, B4	13
	<b>J</b>	M1, I2, I4, I5, I6, B2, B3, B4, Mauthner	I4, I5, Mauthner	12



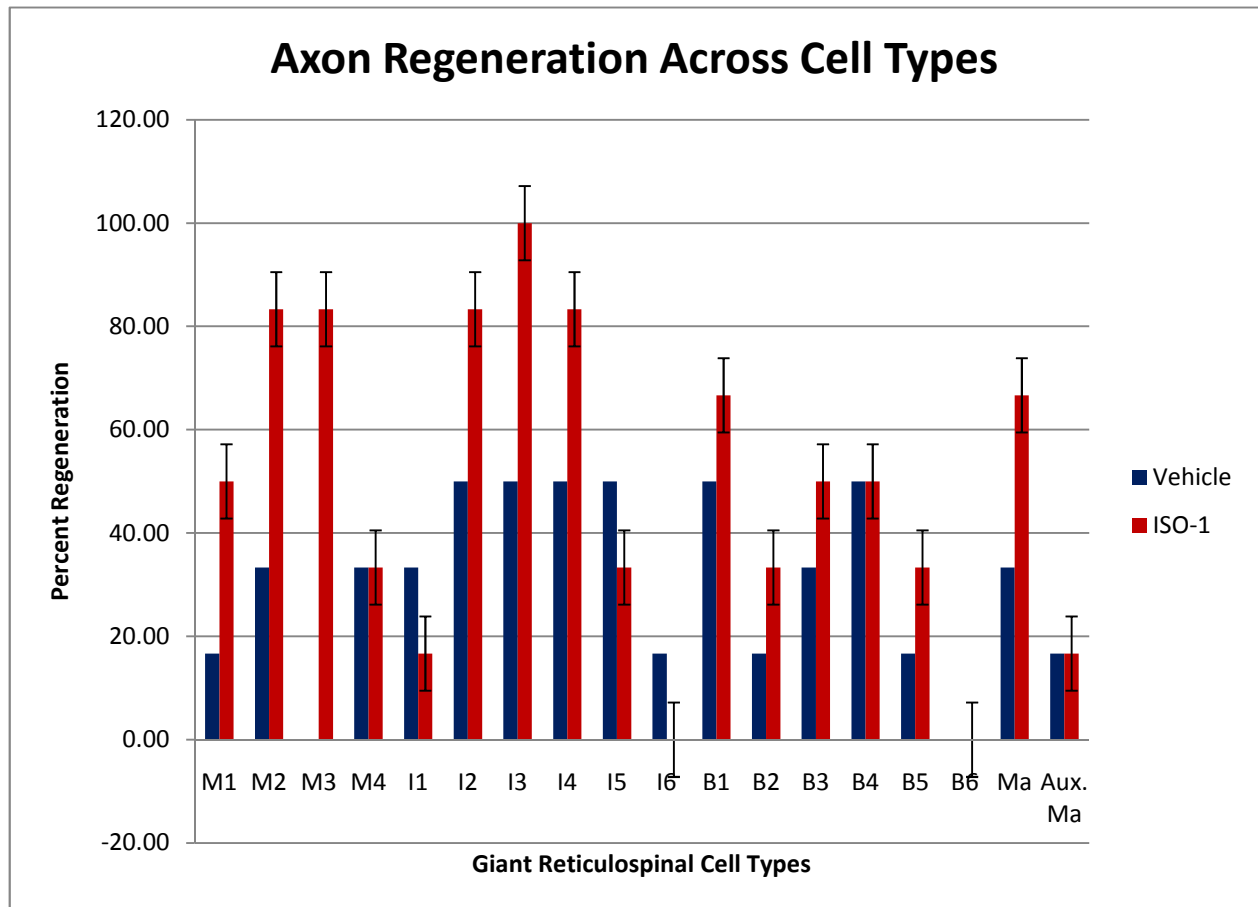
**Figure 8: Average Number of HRP Labeled Neurons**



**Figure 8. Average Number of HRP Labeled Axons.** The lampreys treated only with the vehicle showed on average regeneration of  $11.00 \pm 1.5$  giant RS axons. Lampreys treated with ISO-1 regenerated  $17.67 \pm 1.9$  giant RS axons on average.

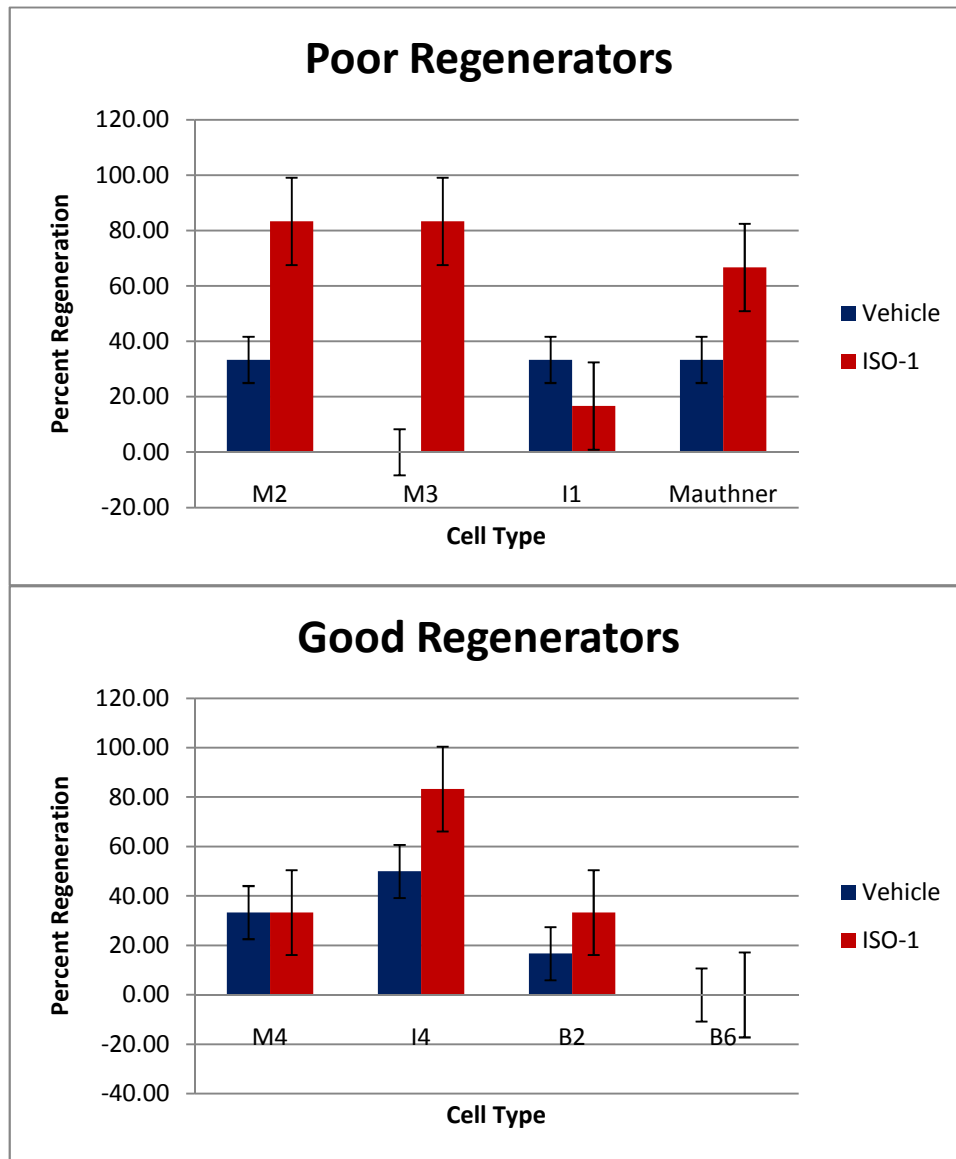
Analysis of the recovered lamprey brains showed a 60.6% increase from the average number of labeled somata of giant RS axons from lampreys treated only with the vehicle to the average number found in lampreys treated with MIF inhibitor (figure 8). ISO-1 treated lampreys had on average  $17.67 \pm 1.9$  HRP-labeled giant RS neurons as compared with vehicle treated lampreys, which contained  $11.00 \pm 1.5$  HRP-labeled giant RS neurons on average. A Student's t-test confirmed a significant increase between the results with  $p < 0.05$ . Because the axons of these labeled neurons were retrogradely labeled from a position caudal to the lesion site, these data strongly suggest an increase in axon regeneration in ISO-1 treated lampreys.

**Figure 9: Axon Regeneration Across Cell Types**



The average percentages of axon regeneration from lampreys treated with and without ISO-1 were calculated according to specific cell type (Figure 9). Once again, lampreys treated with ISO-1 show consistently better axon recovery than their counterparts treated only with the vehicle.

**Figure 10: Good and Poor Regenerators**



Several of the cell types have been identified as consistently “good” versus “poor” regenerators (Jacobs, et al. 1997). The Selzer lab defined good regenerators are axons that have regenerated across the lesion site 100% of the time, whereas poor regenerators generally

regenerate less than 20% of the time. The results found in this experiment for the good regenerators - M4, I4, B2, and B6 – and the poor regenerators – M2, M3, I1, and the Mauthner – were grouped together to determine the existence of any correlations within the categories (figure10). Iso-1 enhanced the number of HRP labeled neurons, regardless of whether the neurons were “good” or “poor” regenerators (figure 10).

## **Discussion**

### **Corroborating Data on the Positive Effects of MIF Inhibition**

The study yielded two sets of corroborating data. On the anatomical level, labeling the cords that regenerated through the lesion site showed that lampreys treated with ISO-1, and thus exhibiting MIF inhibition, resulted in 60.6% more labeled somata than their uninhibited counterparts (figure 8). It is worth noting that the profile of regeneration percentages among specific neurons (figure 9) from the control lampreys, those treated only with the vehicle very closely corresponded to established values (Jacobs et al. 1997). Both the controls in this experiment and the controls in the Selzer study show the best relative regeneration among the I cells, and lower regeneration percentages among the M cells. Overall, the vehicle treated lampreys show lower percentages than the control lampreys in the Selzer study. The regeneration percentages from the ISO-1 treated lampreys, however, show higher percentages than the vehicle treated lampreys across the board but do not correspond to the relative levels of regeneration established by the Selzer lab. There is a possibility that the vehicle may have had a negative effect on regeneration and recovery. ISO-1 had to be dissolved in DMSO, a nonpolar solvent that can damage neurons and tissue. A much higher percent solution of DMSO was used than might be necessary, suggesting that the vehicle may have significantly hindered recovery.

Inhibition of MIF did not largely alter the intrinsic relative regenerative potential of specific neurons, but merely appeared to increase the incidence of regeneration across the board.

What makes the data especially compelling is that the behavioral recovery appeared to correspond quite well with the axonal regeneration. The maximum recovery of the ISO-1 treated lampreys was 11.11% higher than the maximum recovery of lampreys that had been treated with the vehicle alone.

Preliminary results, when taken as a whole, support my hypothesis. MIF inhibition, and thus the curtailing of the inflammatory response, led to more complete behavioral recovery and more effective axon regeneration across the lesion site. It is worth noting that the DMSO vehicle may have been damaging to neurons and tissue, thus adversely affecting regeneration and recovery. In future trials, using a non-destructive solvent or finding an MIF inhibitor that can be more safely administered may in fact yield much higher recovery rates than have been recorded. It would also be necessary to adjust the level of inhibition through dosage of inhibitor in order to determine the most effective level of MIF needed for maximum recovery.

### **MIF and Spinal Cord Regeneration in a Mammalian Model**

While my studies were underway, Nishio et al., 2009 published a finding that corroborates the results of the current experiment in lamprey. In that study, they found that

the inhibition of MIF has a positive effect on neuronal recovery and regeneration (Nishio et al. 2009). Spinal cord injury was induced in MIF knockout mice and wild-type mice, after which locomotive recovery was monitored over a six week recovery period. The knockout mice showed significantly better behavioral recovery than the wild-type mice in assessments of hind-limb motor function. Afterwards, in-vitro glutamate assault showed significantly less death of cerebellar granular neurons in the knockout mice, suggesting the better possibility of recovery on a pathological level. Furthermore, immunohistological studies showed much higher survival of neurons in mice that did not produce MIF. Thus, these data collectively indicate a better survival of neurons after injury when MIF levels are reduced. In light of my results in lampreys, another possible interpretation for enhanced recovery of motor function after SCI in the MIF knockout mice is an increase in axon regeneration across the injury site. Furthermore, the parallels between responses to MIF inhibition in both the lamprey and the mouse model organisms give rise to the possibility of similar responses in higher vertebrates. There is reason to believe that MIF inhibition following spinal cord injury in humans may be beneficial, increasing recovery and enhancing regenerative potential of neurons.

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